



# What is the relationship between the bioaccumulation of chemical contaminants in the variegated scallop *Mimachlamys varia* and its health status? A study carried out on the French Atlantic coast using the Path ComDim model

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## Title

What is the relationship between the bioaccumulation of chemical contaminants in the variegated scallop *Mimachlamys varia* and its health status? A study carried out on the French Atlantic coast using the Path ComDim model.

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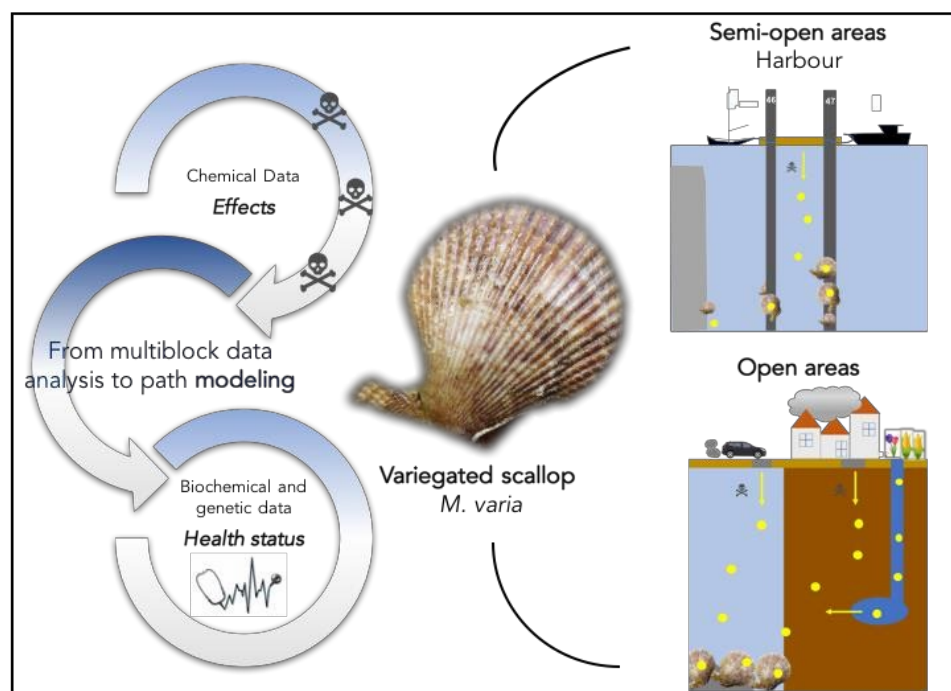
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## Abstract

Increasing activity along the French Atlantic coast has led to chronic pollution with, in particular, mixtures of contaminants such as hydrocarbons, phytosanitary products, PCBs and heavy metals. Based on previous research, pollution biomarkers were used in this study as they can indicate health status when monitoring the impact of pollutants on coastal species such as the marine bivalve *Mimachlamys varia*. Mollusc bivalves were sampled in March 2016, in open and semi-open areas (a harbour zone), from thirteen sites which differed in terms of their level of pollution, and were located along the Atlantic coast from Brittany down to the Nouvelle-Aquitaine region. First, analyses of heavy metals and organic contaminants (e.g. pesticides, polycyclic aromatic hydrocarbons, polychlorobiphenyl) in the digestive gland of bivalves were performed. Second, biochemical assays were used to study defence biomarkers: oxidative stress with Superoxide Dismutase (SOD), detoxification of organic compounds with Glutathione-S Transferase (GST), lipid peroxidation with Malondialdehyde (MDA), and immune processes with Laccase. In addition to the biochemical assays, a genetic approach was used to measure genetic diversity (haplotype and nucleotide diversity) at each site. Biomarker assays and genetic diversity were correlated with the chemical contaminants in bivalves using the Path-ComDim statistical model. Our results showed specific correlations between biochemical assays in the digestive glands with heavy metal contaminants, and between genetic diversity and organic pollution. Blocks of responses were analysed for correlations in order to develop standardized tools and guidelines that could improve our understanding of the short-term and long-term impact of contaminants on physiological parameters.

**Keywords:** *Mimachlamys varia*, chronic chemical contamination, Atlantic coast, genetic diversity, biochemical assays, Path-ComDim model



Integrative approach undertaken in this study to assess effects of a chronic chemical contamination on natural populations of the marine bivalve *Mimachlamys varia*.  
Photo credit: Thierry Guyot.

## Highlights

- *Mimachlamys varia* was shown to be a useful bioindicator for environment quality assessment
- Scallops were collected from 13 areas along the French Atlantic coast, contaminated to different degrees with organic compounds and heavy metals
- Relationships between blocks of variables (contaminants, biomarkers of pollution, genetic diversity) were demonstrated with the Path-ComDim model
- The health of marine bivalves is modulated by polymetallic pollution
- Genetic diversity was negatively correlated to organic contamination

## 1    **1. Introduction**

2            Run-off water containing various chemical contaminants accumulates on the coast.

3    The degree of long-term chemical contamination of coastal waters may vary depending on the  
4    effects of dilution as a result of tides, currents and climate variation. Since the 1970s  
5    (Convention for the Prevention of Marine Pollution from Land-Based Sources, 1974) there  
6    has been a growing awareness of the disastrous consequences of chemical contaminants on  
7    our health and on the well-being of the environment.

8    Aside from the cross-disciplinary policies on issues related to the coast and the maritime  
9    economy, framed by the French National Strategy for the Sea and the Coast and that was  
10   introduced in 2016 (by the Minister for the Environment, Energy and the Sea in August  
11   2016), the regulations concerning the quality of coastal waters are important both at the  
12   national and the European Community level. The regulations pertaining to water quality  
13   define the principles that must be adhered to by European Union member states in order to  
14   achieve a good ecological water status by 2020. These principles are defined in the Marine  
15   Strategy Framework Directive (MSFD), the Water Framework Directive (WFD), in measures  
16   taken by the farming sector (better management of fertilisation, the nitrate directive, the *plan*  
17   *Ecophyto*), and measures taken at the level of catchment areas. The aim is to reduce the use of  
18   phytosanitary, heavy metal, polychlorobiphenyl and hydrocarbon products and to help the  
19   local stakeholders, farmers and port managers to improve the quality of the water.

20   In this context, the aim of the present study was to assess the health of marine bivalves, which  
21   are excellent sentinel organisms for measuring the quality of coastal environments (Grosell  
22   and Walsh, 2006). Additionally, bivalves such as the variegated scallop *Mimachlamys varia*  
23   are commercialised along the French coast. Initial *in situ* ecotoxicological studies were  
24   carried out by our laboratory to assess the presence of pollutants in these organisms  
25   (biomarkers of exposure) and/or to measure the impact of pollutants (biomarkers of effects)

on this species, using both biochemical markers and genetic diversity measurements (Bustamante et al., 2005; Milinkovitch et al., 2015; Breitwieser et al., 2016, submitted-a). Here, a biomonitoring study was performed on 13 sites in the Nouvelle-Aquitaine and Brittany regions in March 2016 (Figure 1). Antioxidant enzyme activity (SuperOxide Dismutase biomarker), detoxication enzyme activity (Glutathion S-transferase biomarker), lipid peroxidation (MalonDiAldehyde biomarker) and immunomodulation (Laccase biomarker) were used as pollution markers. Malondialdehyde and SOD form part of the antioxidant defences and have been used to show the impact of exposure to metals and a wide range of organic compounds in the environment (Amiard-Triquet et al., 2013). Glutathione S-transferases are involved in the phase II detoxification of organic contaminants, and also play a protective role in oxidative stress by catalysing a selenium-dependent glutathione peroxidase (Tappel et al., 1982; Prohaska, 1980). Lipid peroxidation in the MDA assay indicates damage to cellular membrane lipids caused by ROS and is useful for assessing exposure to and the effects of pollutants in marine species (Sureda et al., 2011; Capo et al., 2015). Moreover, in bivalves such as oysters, mussels and variegated scallops, there is a depletion of key enzymes such as laccase, which is involved in the modulation of the immune system in the bivalve in the presence of different kinds of contaminants (Luna-Acosta et al., 2010, 2011; Milinkovitch et al., 2015; Breitwieser et al., 2016). In addition, previous studies on other aquatic organisms have shown that chemical contamination can cause genetic damage at the molecular level and can affect population genetic diversity (e.g. Guttman, 1994; Gardeström et al., 2008). A decrease in genetic variability can result from a reduction in population size due to an increased mortality rate and/or reduced fertility, and from natural selection of individuals with more resistant genotypes (van Straalen et al., 2002; Nowak et al., 2009; Bickham, 2011; Brady et al., 2017). As a result, investigating the genetic effects of



chemical contamination at the population level has been recognized as an important aspect in impact assessment (Bickham et al., 2000).

In contrast to previous work investigating the link between chemical contaminants and genetic diversity in variegated scallops (Breitwieser et al., 2016; Breitwieser et al., submitted-a), the present study is able, for the first time, to relate both inorganic and organic pollutants with genetic diversity measured in the same specimens across a large number of sites along the Atlantic coast. In addition, our study uses an innovative statistical analysis (Path-ComDim) to take into consideration the effect of chronic chemical pollution (pesticides, polycyclic aromatic hydrocarbons, polychlorobiphenyl and heavy metals) on (1) genetic diversity, and (2) the metabolic and physiological response through the combined use of several biomarkers of pollution (SOD, GST, MDA, Laccase) of *M. varia* specimens collected from 13 sites.

This study is an essential step toward monitoring the health of marine coastal environments along the French Atlantic coast. Our work follows the recent implementation of different strategies and operating procedures developed as part of cross-disciplinary policies on issues relating to the coast and the maritime economy, as it provides a measure of environmental quality through the use of a bioindicator marine bivalve.

## **2. Materials and Methods**

### *2.1- Field collection and tissue sampling*

The study sites are shown in Fig. 1. Scallops were collected from 13 sampling sites from Brest down to the Nouvelle-Aquitaine Region. “Loix” was chosen as the less-contaminated site. The open areas, “Troveoc” and “Plougastel”, which are situated in Brest Bay, are subjected to anthropogenic pressure from urban activities and the Elorn estuary. “Erdeven”

and “Auray”, situated in southern Brittany, are subject to heavy contamination from agriculture and pig farming. “Port-Neuf” is situated near a water-treatment plant and the industrial area of La Rochelle. “Angoulins” may be exposed to currents and pollution from adjacent urban tourist areas. “Aix Isle”, “Les Palles” and “Oleron” are situated across the Charente estuary, which is impacted by sources of pesticide pollution, in particular from treatment of the vines.

Finally, for the semi-open areas, “outlet dredging” is an area of outlet dredging near a touristic beach and close to a harbour, “outlet rainwater” corresponds to the outlet of rainwater in a harbour (without a water-filtering system), “outlet fairing” is located below the careening area, which has a water-filtering system (carbon, sand, UV and paper to remove organic, inorganic and bacterial contamination).

Tissue samples from variegated scallops (*M. varia*) were collected from the 13 sites described above. For bioaccumulation and chemical biomarker assays, the digestive glands and gills were sampled from 130 individuals (10 individuals per site) collected in March 2016 (adult size mean of  $4.04 \pm 0.32$  cm). For the genetic approach, muscle tissue was sampled from the same 130 individuals and from additional variegated scallops collected at these sites in 2014 and 2016, to estimate population diversity indices. Thus, genetic data were obtained from 459 individuals (25 to 59 individuals per site) and these data are described in Breitwieser et al., submitted-a).

Sampling and protein assays were carried out in March 2016. For each sampling site, the organs of the bivalves were removed *in situ* and stored at  $-80^{\circ}\text{C}$  for further analysis. Each gill was cut into two samples and examined for trace elements and a biochemical assessment of biomarkers was performed; each digestive gland was cut into two samples in order to assess organic contaminants and perform a biochemical assessment of biomarkers (Breitwieser et al., 2016). Samples for trace element analysis (gills) were freeze-dried for 48

h. For each scallop, muscle tissue was collected and stored in absolute ethanol for genetic analysis.

## 2.2- Assays of heavy metals

Analyses of trace elements were based on previous work by Unrine et al. (2007). Assays of Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, Sn, V and Zn were performed on gills with a Varian Vista-Pro ICP-OES and a Thermofisher Scientific XSeries 2 ICP-MS.

## 2.3- Assays for organic contaminants

The digestive glands of 7 scallops per site were analysed for 21 PAHs, 14 PCBs and 8 pesticides by stir bar sorptive extraction-thermal desorption-gas chromatography-tandem mass spectrometry (SBSE-GC-MS/MS) using a method adapted from Lacroix et al. (2014). The results are expressed as µg analytes/kg dry weight (d.w.). Limits of quantification (LOQ) were calculated using the calibration curve method (Shrivastava et al., 2011) and the limit of detection (LOD) was estimated by dividing LOQ by 3. Analytical quality control was made using the Standard Reference Materials (1974c) “Organics in Mussel Tissue (*Mytilus edulis*)” provided by the National Institute of Standards and Technology (NIST, Gaithersburg, USA).

## 2.4- Biochemical assays

### 2.4.1. Sample processing

For biomarker assessment in gills and digestive glands, samples were homogenized in ice-cold phosphate buffer (100 mM, pH 7.2, 1100 mOsm). The homogenates were centrifuged at 12,500g / 4 °C for 15 min and the Final Fractions (FF) were used for biochemical assays.

#### 2.4.2 Total protein concentration

Total protein concentration was determined using an adaptation of the BCA Kit method (Bicinchononique Acid Kit, Sigma Aldrich). The kit contained bovine serum albumin (BSA) as a standard and involved the reduction of alkaline  $\text{Cu}^{2+}$  by proteins (Smith et al., 1985) at an absorbance of 562 nm using a spectrofluorometer (SAFAS Flx-Xenius).

#### 2.4.3 Antioxidant enzyme activity

SOD activity was determined in the FF using the method developed by Paoletti et al. (1986). The results are presented in U of SOD/mg of protein.

GST activity in the organs was determined by adapting a Sigma assay kit method and the results are expressed in U of GST/mg of protein (Mannervik et al., 1988; Habig et al., 1974).

#### 2.4.4 Laccase assay

Using a method developed by Luna-Acosta et al. (2010), this activity was measured in the FF. The reaction was assessed at 420 nm using a spectrofluorometer (SAFAS Flx-Xenius). In parallel, the non-enzymatic auto-oxidation (oxidation in the absence of FF) was subtracted. Laccase-type PO activity is expressed as U of laccase/mg of protein (one unit is defined as the amount of enzyme that catalyses the appearance of 1  $\mu\text{mol}$  of product per min).

#### 2.4.5 Lipid peroxidation

Damage, and in particular lipid peroxidation, was estimated by assessing the MDA concentration in the final fraction. The product was quantified by measuring absorbance at 586 nm (Gerard-Monnier et al., 1998) using the SAFAS Flx-Xenius spectrofluorimeter. The results are expressed in  $\mu\text{M}.\text{mg protein}^{-1}$ .

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150       2.5-   *Genetic approach*

151       Genetic diversity of scallops was assessed using sequences of a 444 base-pair portion of  
152 the mitochondrial gene coding for cytochrome c oxidase I (cox1). DNA extraction, PCR and  
153 sequencing protocols were described in Breitwieser et al. (2016) and Breitwieser al.  
154 (submitted-a). The haplotype diversity (Hd), and nucleotide diversity ( $\pi$ ) were calculated  
155 using Arlequin version 3.5.2.2 (Excoffier and Lischer, 2010), for each sampling site  
156 separately.

157

158       2.6-   *Data processing*

159       First, preliminary statistical analyses were carried out using R v. 3.1.2 (R Core Team,  
160 2016). In all cases, normality was tested on residuals using the Kolmogorov-Smirnov test and  
161 homogeneity of variance was assessed using the Bartlett test. Moreover, a multi-correlation  
162 analysis was performed to highlight correlations between chemical contaminant  
163 concentrations and biomarker values.

164       Second, a multiblock statistical analysis with a path modelling approach was used to take  
165 into account specific patterns of directed relations among the blocks of variables. In our case  
166 study, five blocks of variables were considered: assays for heavy metals, assays for organic  
167 contaminants, biochemical assays on the gills, biochemical assays on the digestive glands and  
168 genetic data. The advantage of the multiblock data method of analysis used herein, named  
169 ComDim (Mazerolles et al., 2002; Cariou et al., 2017), is to simultaneously exhibit common  
170 dimensions shared by the blocks and introduce a system of weighting that allows each block  
171 to have a specific weight for each common component. Moreover, as reported in Cariou  
172 (2017), the combination of this multiblock analysis with a path modelling approach, known as  
173 the Path-ComDim approach, takes into account the existence of an *a priori* specific pattern of

directed relations among the datasets at hand. Thus, Path-ComDim aims to reduce the dimensionality of data organized in several blocks and to estimate the importance of the assumed effects of one block on another block of information. For this analysis, we used contaminant and biomarker data obtained from 91 of the collected specimens, and the genetic diversity indices obtained for each site (estimated from 25 to 59 individuals per site) (Figure 2).

We considered a path diagram with arrows directed from heavy metals to biochemical assays in the gills, digestive glands and the genetic data on the one hand, and organic contaminants to biochemical assays in both organs and genetic diversity, on the other (Figure 2). These arrows reflect the fact that the chemical contamination measurements (heavy metals and organic contaminants) are assumed to have an influence on the health status measured by the biochemical assays and the genetic approach.

From a practical point of view, each block of data was subjected to a pre-treatment: at a given site, organic contaminants for which assay values were regularly under the LOQ (except for two or three observations) were summed. Thus, several PAH variables were aggregated into a variable named “PAHothersum”, and several PCBs were aggregated into the variable “PCBothersum”. All eight pesticides were also summed together. Afterwards all the variables were individually standardized and each block was set at the same total variance to account for the fact that the number of variables is different from one block to another.

### **3. Results**

Data used in the Path-ComDim analysis are shown in the supplementary data (Tables S1 and S2, Figure S1). For this analysis, we used heavy metals contaminants (Figure S1), organic contaminants concentrations (Table S1), the genetic diversity indices obtained for each site (Table S2) and biomarker data (Table S3).

Table 1 illustrates the results of the first three common components, obtained using Path-ComDim. It turns out that the first two components (dim. 1 and 2) have an overwhelming importance (47.9 % for dim. 1 and 34.5 % for dim. 2) in comparison to the third component (7.0 % relative importance). Table 1 also shows the contribution of each pair of linked blocks of variables to the definition of each common component. It appears that the first common component (dim.1) mainly highlights the link between organic contaminants and the genetic data (99.3%). The second common component mainly highlights the link between heavy metals and biochemical assays in the digestive gland (83.8%), whereas a weak relationship was observed between organic contaminants and biochemical assays in this organ (8.6%). The links between heavy metals and biochemical assays in the gills, between heavy metals and genetic diversity and between biochemical assays and organic contaminants in the gills do not really contribute to the two first common components.

The third component mainly accounts for the link between heavy metals and biochemical assays in the gills, but as the relative importance of this dimension is very small (0.4 %), this result will not be taken into account in the following analysis.

From now on, we shall focus on the first two components, denoted t1 and t2. Figure 3 shows the configuration of the samples, in relation to their respective site, on the basis of t1 and t2. Figure 3 also shows correlations between the common components t1 and t2 and the variables according to the block they belong to (heavy metals, organic contaminants, biochemical assays in the gills and digestive gland, genetic approach). As each common component is built to take account as much as possible of the relationship between the blocks of variables, the subplots 3b to 3f in Figure 3 make it possible to illustrate the links between the blocks of variables depicted in Table 1.

Concerning the block of heavy metal variables (Figure 3b), three clusters of heavy metals can be observed along the second component, t2, namely Cu/Se/Zn/As, Cd/Ag, and

Pb/Cr/Co/Fe/Mn/Ni. This partition was confirmed using a clustering approach for variables (Vigneau et al., 2015). The Sn and V values were not taken into account because of the lower quantification with respect to the LOD value. The three clusters of heavy metals seem to be associated with location. Indeed, the sites “Outlet rainwater” and “Outlet Fairing”, which are situated in a harbour area, could modulate the Cu/Se/Zn/As cluster, whereas “Outlet dredging” and the majority of the sites situated near the Charente Estuary (“Oleron”, “Angoulins”, “Port-Neuf”, “les Palles”) could influence the Pb/Cr/Co/Fe/Mn/Ni cluster.

Moreover, it was interesting to show (Table 1) the strong contribution link between heavy metals and biochemical assays in the digestive glands. Indeed, the Path-ComDim model showed that the biochemical values for the digestive gland had a common dimension (were of a similar order of magnitude) on axe 2 (Figure 3e). In the digestive glands, there was a positive correlation between the biomarkers GST and MDA, but a negative correlation between the group GST/MDA and the Laccase biomarker. The SOD biomarker involved in oxidative stress did not correlate with the other pollution biomarkers (laccase, MDA, GST).

The organic contaminants (pesticides, hydrocarbons, polychlorobiphenyls) are well depicted along the common dimension t1. The correlation plot on Figure 3c shows that the Polycyclic Aromatic Hydrocarbon (PAH) variables (except biphenyl), several PCB variables (PCB28, PCB77 and the sum of the rare PCBs) and the variable summing all the pesticide assays correlated well (negatively) with t1. All organic contaminants are situated to the left-hand side of the plot, and in greater quantity in the “Plougastel” site (for pesticides and a rare PCB group named “PCB other sum” in Figure 3c), “Outlet fairing”, “Outlet rainwaters” and “Outlet dredging” sites concerning PCB 28, PCB 77 and all hydrocarbons (Figures 3.a and 3.c).



The two genetic variables on Figure 3f (haplotype diversity and nucleotide diversity) are positively correlated with t1 and are expressed more in sites such as “Loix”, “Angoulins”, “Les Palles” and “Oleron” on the right-hand diagram (Figure 3a). The genetic variables are not correlated with t2 and thus seem not to be linked with heavy metal contaminants (Figure 3b). In contrast, t1 illustrated the negative relationship between organic contaminants (Figure 3c) and the genetic block (Figure 3f). The greater the quantity of organic contaminants, the lower the indices of genetic diversity (haplotype diversity, nucleotide diversity). This link contributed greatly to t1, as already shown in Table 1.

It is interesting to note that the results of the biochemical assays on the digestive glands (Figure 3e) were different to those in the gills (Figure 3d). Indeed, it seems that there was a significant background noise in the results of the biochemical assays on the gills. As a consequence, the block of biochemical assays on the gills appeared to have no strong links with the other blocks.

These analyses are particularly interesting in comparison with heavy metals, which had a link contribution of 83.8% on common dimension 2 (Table 1). Indeed, a greater contamination of Co, Cr, Pb and Ni led to a positive immunomodulation in the “Outlet dredging” site, whereas a decrease in the specific activity of laccase was influenced by As and Se at “Plougastel”. Moreover, the higher contamination with As and Se induced a higher MDA and GST response, both of which are involved in lipid peroxidation and the detoxification of xenobiotic compounds, unlike laccase.

#### **4. Discussion**

In this work, an innovative analysis of the effects of chemical contamination (inorganic and organic pollutants) on the biology of the variegated scallop *M. varia* was carried out using

a statistical approach involving the Path-ComDim model to: i) clearly study responses to both contaminants (heavy metals and organic pollutants); ii) identify the biochemical and genetic effects for each type of contaminant; and iii) understand the combined effect of the contaminants on health status.

#### 4.1- Chemical contaminants

Our study was carried out using scallops collected *in situ* in March 2016, and for each specimen, the level of contamination with heavy metals and organic pollutants was estimated in the gills and digestive glands. A small number of bio-monitoring investigations of coastal waters have been carried out by the local stakeholders on the Atlantic coast to evaluate the environment quality (e.g. the water department of the local authorities, the harbour authorities). Using the variegated scallop *M. varia*, our results showed higher concentrations of Pb, Cr, Fe, Ni, Co in the gills of this bivalve in the Pertuis Charentais (“Oleron”, “Angoulins”, “Port-Neuf”, “Les Palles” sites) and in particular in “Outlet dredging” zones in La Rochelle harbour in comparison with the least-contaminated area, “Loix”, described as a reference site in the literature (Bustamante and Miramand, 2005). Moreover, Cu and Zn concentrations were higher in “Troveoc”, “Erdeven”, “Auray” and “Outlet rainwater” than in “Loix”. These results confirm the hypothesis that the Atlantic coast is contaminated by trace elements from anthropogenic activities in wastewaters and inputs from drainage areas directly adjacent to the sea, and watershed-derived inputs (e.g erosion of contaminated land, effluents from highly-urbanized rivers, domestic and/or industrial effluents from wastewater treatment plants, urban runoff and sewers). Furthermore, the capacity of heavy metal contaminants to bio-accumulate in bivalve molluscs (Bryan, 1973) depends on the main function of each organ i.e. trapping nutrients (gills), or digesting and excreting nutrients (digestive gland). Indeed, previous studies have shown a greater bioaccumulation of chemical contaminants in

the digestive gland in comparison with the gills (Breitwieser et al., submitted-b). However, it is important to underline the difference between non-essential and essential metals: non-essential heavy metals such as As, Pb, Co, Cd could be more toxic for the scallop (Rainbow et al., 2002) and on a larger scale, for Human. In fact, these trace elements are used particularly in industrial sectors, wood preservatives, electrical battery manufacturing, alloy production, and are classified in the first group of carcinogenic compounds (International Agency for Research on Cancer). According to a report by the National Institute for Public Health (Tome 2: heavy metals and metalloids, December 2017), their major impregnation by human could be due to seafood consumption.

Organic contaminant levels in the digestive gland of *M. varia* scallop provided information on anthropogenic sewage in 13 separate sites on the Atlantic coast. In this work, the data obtained showed high levels of pesticides in Plougastel (sum of dieldrin, aldrin, isodrin, endrin, 2-4 dde, 4-4 dde and rare PCBs (52, 101, 135, 118, 153, 105, 138, 156, 180, 169)) collected together in a single variable (Figure 3c). This might be explained by the slow and insidious degradation of these components, and in particular PCBs, which are now prohibited because they are responsible for acute toxicity. Concerning PCBs 28 and 77, measurements in the digestive glands of *M. varia* indicated that the mean of these two PCBs was significantly higher in Plougastel, outlet rainwater and fairing areas. Moreover, measurements of pyrene and other hydrocarbon congeners in the digestive glands showed anthropogenic pressure (S1 Table of supplementary data). These results might be due to the proximity of urban zones along the Atlantic coast, despite the fact that all of these contaminants are still considered as a priority by the WFD in France.

Indeed, on the correlation plot on Figure 3c, all the contaminants are situated on the left of the plot in the common dimension 1. All the organic components could potentially originate from various activities in these sectors (fishing harbours, tourist harbours, petroleum

refinery, waste treatment plant) with tidal currents, particularly from the Gironde Estuary and from Brittany (Luna-Acosta et al., 2017). Moreover, previous research indicated that the concentration of pesticides, PCBs and PAHs in sediments was higher than in the surrounding water, and this might account for the observation in the digestive glands of *M. varia*, which is a sedentary benthic species (Luna-Acosta et al., 2017).

These data are in agreement with previous results showing that other marine bivalves (mussels, oysters and scallops) collected on the Atlantic coast have a strong ability to ingest and bio-accumulate more hydrophobic high molecular weight PAHs and heavy metals in their digestive organs than in other organs as gills and mantle (Luna-Acosta et al., 2017; Breitwieser et al., 2017, Lacroix et al., 2017).

#### 4.2- Health status response

Because benthic marine molluscs are exposed to multiple stressors such as contaminant cocktails and it is not always possible to test for their presence within tissues, there has been a search for indicators that demonstrate the toxicity of chemical pollutants. The advantage and originality of our study is that indicators of health status and contaminants were assessed using the Path-ComDim statistical model in distinct tissues. As previously suggested in *M. varia*, indicators of health status are pollutant biomarkers for the short-term (GST, MDA, SOD, Laccase). It is for this reason too that biomarkers are currently being used to assess the condition of European coastal waters (Hagger et al., 2009).

From the results obtained with the Path-ComDim model, the biomarkers in the gills of the variegated scallop were not modulated, in contrast to the biomarkers in the digestive gland and the genetic diversity indices. This may be linked to the fact that the ability to deal with oxidative stress, detoxification and immunomodulation revealed by pollution biomarkers may depend on the accumulation and the kind of contamination (Hannam et al., 2010).

In this work, the biomarkers of oxidative stress used were SOD and MDA. It was demonstrated that these indicators could provide some of the first signals of stress in molluscs (Regoli et al., 2000; Valavanidis et al., 2006). Furthermore, GST is a phase II conjugation enzyme that intervenes in the metabolism of xenobiotics and in reactions which are catalysed by membranes, or cytosolic enzymes. According to previous studies, GST-specific activity increases in exposed organisms in relation to the concentration of xenobiotics in the medium. Indeed, GSTs, which correlated positively with MDA in this study, are enzymes whose activity is used as a biomarker of exposure to organic substances, especially in molluscs, where EROD activity (a marker of cytochrome P450 involved in the metabolism of potentially toxic compounds) is not routinely assessed (Cajaraville et al., 2000). Laccase proved to be a sensitive biomarker and phenoloxidases seem to play an important role in bivalve immunity when they are activated by ROS (Luna-Acosta et al., 2010). Moreover, Laccase contains metal coenzymes which can be affected by inorganic contamination (heavy metals) in a similar fashion to the activation pathway of SOD in the short term, which explains the contrasting response of this enzyme activity to that of the other biomarkers, GST and MDA.

We proposed to use genetic diversity as an indicator of population health because populations with high levels of genetic variation tend to have a better viability (Frankham et al., 2002), a better capacity to resist environmental stress (e.g. Nowak et al., 2012) and possess a greater adaptive potential when facing environmental changes (Eizaguirre and Baltazar-Soares, 2014). Since historical processes and dispersal (i.e. gene flow) influence the genetic diversity of populations, information on the species phylogeography and population structure is required to interpret differences in genetic diversity across sampling sites (Theodorakis, 2003). With that goal, we recently assessed the genetic structure of *M. varia* using *coxI* sequences and did not detect genetic differentiation among sites along the French

coasts of the Bay of Biscay and English Channel (Breitwieser et al., submitted-a). Results based on this mitochondrial DNA marker suggest that all individuals sampled in the present study belong to the same population and that differences in genetic diversity observed among our sampling sites (Supplementary Table S2) are not due to genetic drift or historical events.

Different regions of France and many countries of the European Union have established an integrated ecosystem-based approach to the management of marine resources. Any assessment of the cumulative short-term or long-term effect of all the activities that can potentially impact an environment that is prone to large natural variations is a relevant but difficult task. In recent years, pragmatic approaches have been initiated by urban authorities, and local and regional stakeholders of Charente-Maritime (France). The aim of these voluntary actions is to monitor quantitative chemical concentrations but not to study the qualitative effects on environmental quality. Today, several pollutant biomarkers are being employed in biomonitoring studies but the results are difficult to compare. This is why our research was focused on refining and standardising current biomarker techniques for specific contaminant types using a statistical model as this will allow comparisons to be made.

#### *4.3- Relationship between health status and chemical contaminants*

Thanks to the Path-ComDim model, a comprehensive analysis of our results could be performed. Based on previous research, the discussion will be divided into two parts to explain the relationship between health status and contaminants in this study. In contrast to others studies (Breitwieser et al., 2016), all heavy metals and organic pollutants were considered in the model because all of the data come from the same 91 specimens, which is a relevant approach.

Concerning biomarkers of pollutants, only the results in the digestive gland showed a significant response with heavy metal contaminants. Our model accounts for 83.8 % of link

contribution, suggesting that MDA, which reflects lipid peroxidation, and GST, which shows detoxification of xenobiotics, were positively correlated and were modulated by Cu, Se, As and Zn. Furthermore, based on the model, these four heavy metals, and a group of specific organic contaminants (pesticides, and PCBs 28 and 77), could significantly diminish Laccase activity, in agreement with previous work (Luna-Acosta et al., 2017; Luna-Acosta et al., 2015; Milinkovitch et al., 2015). These results could explain the high functionality of the xenobiotic bio-transformation process by GST on the one hand, and also the decrease in antioxidant protection and immune modulation by an increase in MDA and a decrease in Laccase on the other hand (Pan et al., 2005; Pan et al., 2009; Breitwieser et al., 2016). These enzyme-specific activities and concentration levels could be related to the high contaminant concentrations in the harbours, outlet rainwater and touristic areas (Breitwieser et al., 2017). However, it should not be forgotten that abiotic factors could modulate biomarker activities and bioaccumulation. Indeed, data on physico-chemical parameters collected at the same date are shown on a map of work presented in Breitwieser et al., (submitted-a). Only a significant difference in salinity was discernible between Erdeven, Auray and all other sites, but these two sites are mixed with others on the plot of sites (Figure 3a).

Concerning genetic effects of pollution, it has already been stressed that chemical contaminants can reduce genetic diversity (e.g. Nowak et al., 2009), or induce deleterious mutations that can be heritable (e.g. Cronin and Bickham, 1998), and per consequence, can impact the population health (Bickham et al., 2000; Bickham, 2011). However, some studies have observed greater genetic diversity in polluted environments (Pedrosa et al., 2017) illustrating that the relationship between chemical contamination and genetic diversity is not straightforward and will depend on the species demography, dispersal, and level of contamination.

In our study, low levels of genetic diversity were clearly correlated with high levels of organic contaminants in variegated scallops from outlets fairing, dredging and Plougastel. Since gene flow does not seem to be restricted among our sampling sites (Breitwieser et al., submitted-a), a reduction in genetic diversity at more polluted sites cannot be explained by a population bottleneck. Conversely, selective mortality of individuals possessing mitochondrial haplotypes susceptible to organic pollutants is plausible. Indeed, mitochondria seem to be particularly vulnerable to environmental contaminants (Meyer et al., 2013 and references therein), therefore natural selection may be acting on the mitochondrial genome in organisms exposed to chemical contaminants.

To further test our hypothesis, extensions of this study could include i) obtaining genetic diversity estimates from neutral markers (nuclear loci not under natural selection) and conducting a more fine-scale investigation of gene flow among sites using multiple independent genetic markers such as microsatellites, and ii) assessing experimentally differential mortality of distinct mitochondrial haplotypes following organic contaminant exposure. Additionally, genome-wide data could be used to investigate local adaptation to pollution (Oziolor et al., 2017), which may occur despite gene flow (Tigano and Friesen, 2016). Finally, our study focused on the adult stage *in situ*, but chemical contamination also affects early life stages (larvae and juveniles) in bivalves (e.g. Calabrese et al., 1977). Thus, a multigenerational controlled experiment submitting variegated scallops to chemical contaminants, and measuring mortality and genetic diversity of the different life-stages would provide further insights on the resistance and potential adaptation of this bioindicator species to contamination.

Overall, the statistical model used for monitoring health status is relevant. Indeed, contrary to other models such as PLS-Path Modelling (Tenenhaus et al., 2005; Bertrand et al., 2017) which extract one dimension by block and fit a unique common model, the Path-



ComDim model produces several common dimensions, each of them defining specific weights for the blocks with regard to the links investigated. Furthermore, the graphical output of Path-ComDim can be read like a principal component analysis (PCA). This leads to rather simple and interpretable media for the scientific community.

## 5. Conclusion

In conclusion, the maritime economy serves a wide range of diversified activities, both traditional (fishing, shell fishing, harbour activities, tourism etc.) and emergent (renewable marine energy, biotechnology etc.). In addition to these activities, which are the mainstay of the maritime economy, indirect activities such as logistics, industrial port zones and transport have to be considered. In France, and in particular in the majority of our study sites, the south Atlantic seaboard, with its 720 kilometres of coastland and the Charente-Maritime islands, is an attractive space. Out of interest, the population is constantly growing in the French South-Atlantic, at a rate of 9.5% between 1999 and 2011, in comparison with the population growth in France, which was 7.9% during the same interval.

In recent years, shellfish farmers have experienced repeated crises, and this has raised serious issues. France is the biggest oyster producer and the third biggest mussel producer in Europe according to the “Comité National de la Conchyliculture, <http://www.cnc-france.com>”. Apart from the health risk, a decrease in the quality of the water, the living environment of marine species, would damage marine environments, biodiversity and the development of economic stakeholders (the aquaculture sector, fishing and also tourism). So, it is now crucial to include an assessment of coastal water quality no less frequently than annually. Indeed, using new tools such as Path-ComDim applicated in this study, it is now necessary to address coastal water contamination; this has been undertaken by some harbour

authorities, in particular in La Rochelle, which created a Department of the Environment 10 years ago.

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652

**Table 1:** Importance of the three first common components of Path-ComDim and the contribution of each pair of kinked blocks to the common components.

Importance of the common components			Contribution of each link (%)						
dimension	Absolute importance	Relative importance for each dim.	→	Heavy metals to BCH gills	Heavy metals to BCH dg	Heavy metals to genetic approach	ORG to BCH gills	ORG to BCH dg	ORG to genetic approach
1	0.0080	47.9%	→	<0.1%	<0.1%	0.5%	<0.1%	0.1%	<b>99.3%</b>
2	0.0057	34.5%	→	0.3%	<b>83.8%</b>	5.2%	<0.1%	8.6%	2.10%
3	0.0012	7.0%	→	<b>96.8%</b>	2.6%	<0.1%	0.5%	<0.1%	<0.1%

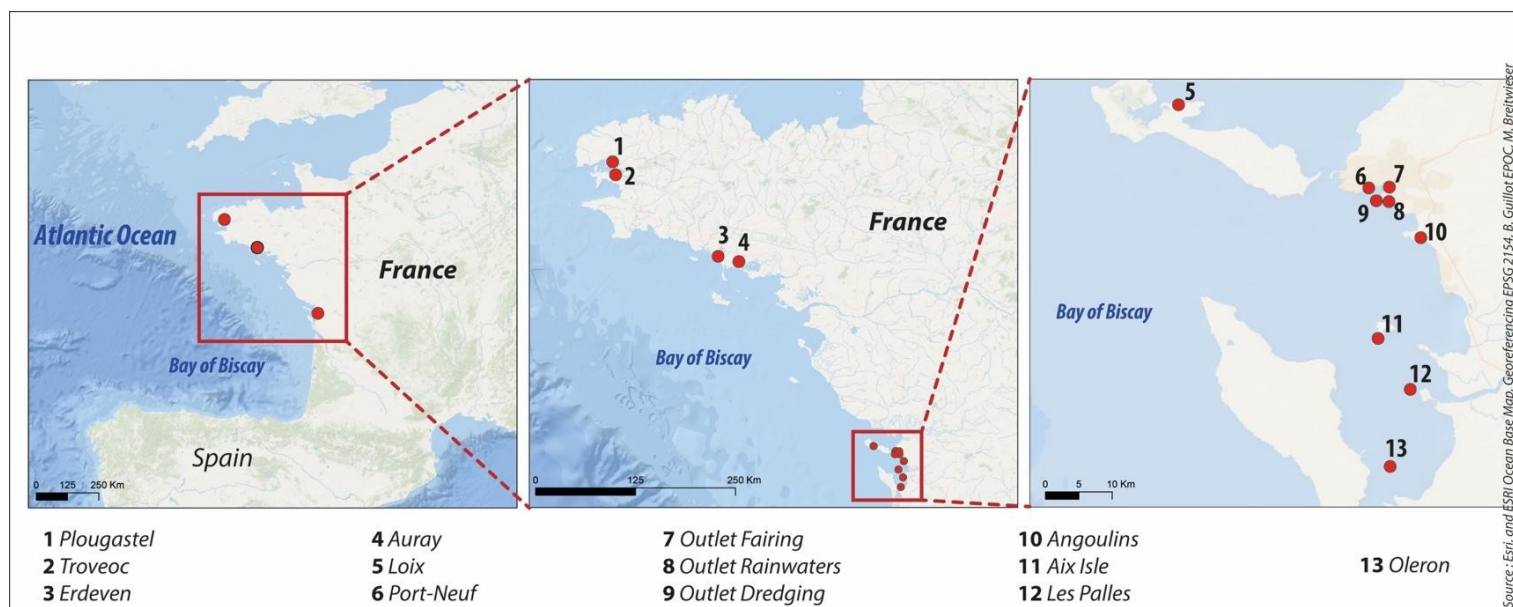
**Caption for table 1:**

*BCHdg: biochemical assays in the digestive glands*

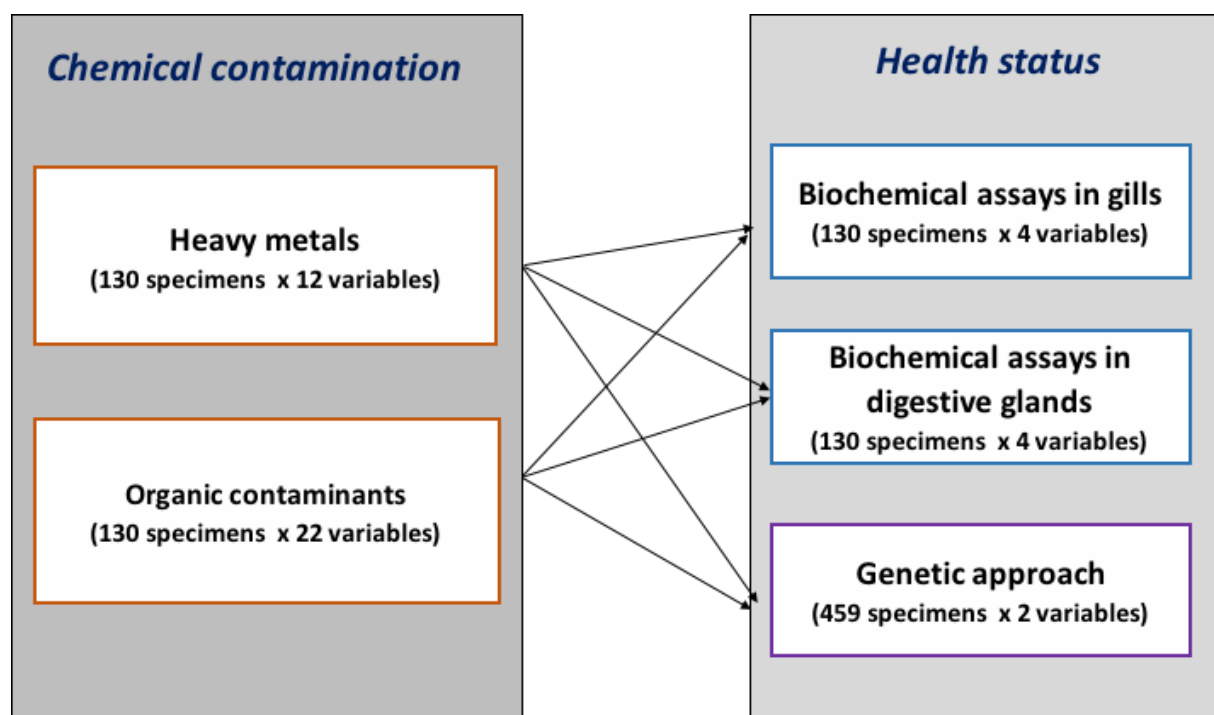
*BCHgills: biochemical assays in the gills*

*ORG corresponds to the organic contaminants*

*Values in the first part of table (importance of the common components) were evaluated as the sum of squares of the saliences of the blocks to the common component extracted at each step (i.e. dimension)*



**Figure 1:** Map of France with the location of the study sites in the Brittany and Nouvelle-Aquitaine regions.



**Figure 2:** Path diagram for the five data blocks report summarizing chemical contamination with two blocks (heavy metals and organic contamination) and health status with three blocks (biochemical assay in two organs and the genetic approach). For each block, we analysed the same 130 specimens for bioaccumulation and biochemical aspects and 459 individuals (25 to 59 per site) for genetic study of the variegated scallop *Mimachlamys varia*.



